REMARKS

In the Office Action dated January 20, 2010, claims 45-46, 50-54, 60-61, 63-65, 68-71, 87-89 and 91 were pending. Claims 63 and 91 were withdrawn from consideration as directed to non-elected subject matter. Claims 45-46, 50-54, 60-61, 64-65, 68-71 and 87-89 were under consideration. Claims 51 and 60 were rejected as allegedly indefinite under 35 U.S.C. §112, second paragraph. Claims 45-46, 50-54, 60, 64-65, 68-71 and 87-89 were rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Amit et al (*Developmental Biology* 227: 271-278, 2000) in view of Mummery et al. (*Differentiation* 46: 51-60, 1991), Rohwedel et al (*Cells Tissues Organs* 165: 190-202, 1999 (Abstract), and Rohwedel et al (*Dev Biol* 164(1): 87-101, 1994).

35 U.S.C. §112, Second Paragraph Rejection

Claim 51 is rejected as indefinite because of the phrase "visceral endoderm-like".

Applicants respectfully submit that those skilled in the art would understand this phrase to mean that the tissues or cells in question have most of the characteristics of visceral endoderm tissue or cells, but are not visceral endoderm tissues or cells *per se*. The characteristics of visceral endoderm are clearly disclosed in the specification, e.g., on page 10, line 29-30; page 11, lines 1-10. Further, as evidenced by Mummery et al. (*Differentiation* 46: 51-60, 1991), visceral endoderm-like cells are understood to mean cells that express marker proteins characteristic of the differentiation of this early lineage, including alpha-feto protein which is expressed in all END2 cells and about 50% of EPI-7 and PSA-5E cells (also VE-like cells). Additional markers such as FT-1 are described in a number of papers (including Mummery et al. 1991). Therefore, claim 51 is not indefinite. New claim 133 is also added to

further define the cell which is derived from VE-like tissue to express alpha-feto protein, as supported by the specification on page 11, lines 1-10, for example. No new matter is introduced.

Claim 60 is also rejected as indefinite because of the recitation, "substantially". Applicants respectfully submit that a "substantially confluent" monolayer is clearly understood by the skilled artisan as a layer of cells that *almost* completely, or completely covers the culture dish surface. Those skilled in the art who are familiar with cell culture would understand the term "substantially confluent" without ambiguity.

In view of the foregoing, Applicants respectfully submit that claims 51 and 60 are not indefinite. Withdrawal of the rejection under 35 U.S.C. §112, second paragraph is overcome.

35 U.S.C. §103(a) Rejection

Claims 45-46, 50-54, 60, 64-65, 68-71 and 87-89 were rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Amit et al (*Developmental Biology* 227: 271-278, 2000) ("Amit") in view of Mummery et al. (*Differentiation* 46: 51-60, 1991) ("Mummery"), Rohwedel et al (*Cells Tissues Organs* 165: 190-202, 1999 (Abstract)) ("Rohwedel (1999)"), and Rohwedel et al (*Dev Biol* 164(1): 87-101, 1994) ("Rohwedel (1994)").

Examiner's Prima Facie Case

Amit allegedly teaches co-culture of human embryonic stem cells (hES) plated on irradiated mouse embryonic fibroblasts (MEFs). The Examiner admits that Amit does not teach a co-culture of END-2 cells with human embryonic stem cells (hES). However, the Examiner relies on Mummery, which teaches co-culture of P19 embryonal carcinoma (EC) cells with END-2 cells. Mummery teaches that when P19 cells were co-cultured with cells

from one of several established visceral-endoderm-like cells lines, the EC cells were rapidly induced to aggregate and differentiate into cell types including mesoderm-derived cardiac and skeletal muscle.

The Examiner also refers to the teaching of Mummery relating to the addition of 10^{-9}M retinoic acid (RA) into charcoal stripped-FCS supplemented media, which media rendered the cells sensitive to the inducing action of retinoic acid, forming beating muscle. In this connection, the Examiner additionally refers to two Rohwedel articles - Rohwedel (1999) teaches induction by retinoic acid of cellular differentiation of pluripotent EC and ES cells *in vitro*; and Rohwedel (1994) teaches that the mouse ES cell line, BLC6, efficiently differentiates into skeletal muscle cells when cultivated in embryo-like aggregates. The effect of external signals on myogenic differentiation of BLC6 cells was demonstrated by co-cultivation with visceral endodermal END-2 cells and the activin A-secreting WEHI-3 cells.

Therefore, the Examiner concludes that it would have been obvious to the ordinarily skilled artisan to modify the co-culture system of Amit by utilizing END-2 cells instead of MEFs to co-culture with hES cells, as taught by Mummery, with a reasonable expectation of success. The Examiner contends that one of ordinary skill in the art would have been motivated to make this modification in order to induce cellular differentiation of hES cells by retinoic acid (RA), as suggested by Rohwedel et al. (1999) where pluripotent EC and ES cells were induced to differentiate by RA *in vitro* and where both cellular systems are suitable to study differentiation of various cell types, because they recapitulate early stages of mouse embryogenesis.

Applicants respectfully disagree, and submit the following in traverse.

Amit

Applicants respectfully submit that the Examiner's reliance on Amit is misplaced. Amit teaches the use of an embryonic cell type (mouse embryonic fibroblasts) to maintain the hES cells in an undifferentiated state. The Examiner notes that if this embryonic cell type is not included, even in the presence of the combination of LIF and FGF, the human ES cells are "still lost to differentiation". Therefore, the teaching of Amit is directed to the use of an embryonic cell line to prevent differentiation of hES cells.

In contrast, the use of the embryonic cell type in the present invention is to cause the differentiation of the hES away from their undifferentiated, pluripotent state and direct differentiation toward mesoderm and the formation of cardiomyocytes and vascular endothelial cells. Therefore, in the context of the present invention, an embryonic cell type (such as visceral endoderm or visceral endoderm-like or END2) is used to achieve efficient differentiation toward a particular germ layer lineage (mesoderm) and subsequent differentiated cell types.

As the Examiner does note, Amit does not teach a co-culture of END2 cells with human ES cells. Consistent with the above submission, END2 cells are used in the present invention to achieve differentiation of human ES cells rather than to prevent differentiation.

Therefore, it is improper for the Examiner to rely on Amit as a primary reference and to combine with additional references in rejecting the instant claims, because Amit does not provide any motivation, and in fact teaches away from the present invention, as discussed.

Mummery et al (1991) and Rohwedel et al (1999)

Mummery teaches that co-culture of mouse EC cells (P19) with a cell line of visceral endoderm-like characteristics (such as END2) resulted in differentiation of PC19 cells

to beating cardiomyocytes.

As Applicants submitted previously, those skilled in the art would not have expected the teachings of Mummery, which relate to differentiation of a mouse EC cell line such as P19, to apply to differentiation of human ES cells, given the clear differences that exist between mouse ES and EC cells and between human EC and ES cell lines. The Examiner's attention is respectfully directed to Applicants' Response dated October 26, 2009, for example, pages 9-13 for detailed discussion in this regard. The distinctions between the cell types are further addressed below by reference to the differing effect of retinoic acid (RA) on mouse and human ES cells, another potent differentiation agent which the Examiner has noted from the referenced prior art (Mummery and Rohwedel (1999)).

As described by the Examiner, the teaching of Mummery shows that when DCC-FCS (charcoal stripped serum) is used with P19 cells in hanging drop culture, cardiomyocytes are efficiently formed. The differentiative effect of RA on mouse EC and ES is widely known (as reviewed by Rohwedel (1999)). As shown in Mummery, stripping the serum of liphophilic compounds (such as RA) appears to make P19 cells grown in this depleted media more sensitive to the effect of supplemented RA at the doses provided. However, the observation of the differentiating effect of RA, discussed in Mummery and in Rohwedel (1999), does not have any relevance to the use of an embryonic cell type (such as visceral endoderm-like cells, e.g., END2 cells) or the effect such cell may have on differentiation of PC19 cells toward cardiomyocytes and vascular endothelial cells.

Consistent with Applicant's previous Response, the effect of differentiation agents on mouse ES and EC cells is different from that on human ES and EC cells, and the outcome for human ES cells would have been unpredictable based on the experimental response of

mouse EC and ES cells. In support of this position, Xu et al. (2002) (previously submitted with Applicants' Response dated October 26, 2009, which shows the differing effect of DMSO on mouse relative to human EC and ES cells) report that RA does not have the same effect on human ES cells as it does on mouse P19 cells. See, e.g., page 504 of Xu et al. (attached again herewith) in the section entitled "*Effect of differentiation Inducing reagents on cardiomyocyte differentiation*", where it is stated:

> "In order to enhance cardiomyocyte differentiation, the effect of differentiation induction reagents was evaluated. DMSO and RA which have been shown to induce cardiomyocyte differentiation in mEC P19 cells and mES cells, respectively, were evaluated but did not enhance hES cell cardiomyocyte differentiation (additional results in the online data supplement)".

In the online data supplement to this paper (also provided herewith), it is stated:

> "We also evaluated retinoic acid (RA), a reagent that enhances cardiogenesis of mES cells and expression of cardiac α-actin in hES cells. RA was added to hES cell cultures at different doses and different times of differentiation (day 0 to 4, 4 to 8, 8 to15 and 4 to15). Treatment with 10^{-9} –10^{-5} M RA at day 0 to 4 was toxic to the cells and did not improve cardiomyocyte differentiation when added to the culture at later times of differentiation. Therefore, DMSO and RA did not enhance H1 hES cell cardiomyocyte differentiation, in contrast to the positive effect these compounds have on mEC and mES cardiomyocyte differentiation, respectively."

Therefore, the effect of RA on human ES cells would not have been predictable and therefore not obvious from the study of the effect of the compound on mouse ES or EC cells.

Rohwedel (1994) and Rohwedel (1999)

The example from Rohwedel (1994), cited by the Examiner, teaches that the mouse ES cell line BLC6 efficiently differentiates to myocytes and skeletal muscle, a mesoderm derivative, when cultured as embryoid bodies. The Examiner also states that an effect of END2 on the myogenic differentiation of the mouse BLC6 ES cell lines was shown by co-cultivation with END2 and the presence of Activin A secreted from the WEHI cell

line. Notably, this disclosure of Rohwedel (1994) only shows an effect of END2 co-culture on the formation of myocytes and skeletal muscle cells from mouse ES cells, rather than the differentiation to cardiomyocytes or vascular endothelial cells from human ES cells as claimed in the present invention.

It was also noted in the Rohwedel (1994) study that the BLC6 ES cell line is particularly suited to the study of skeletal muscle development, as this mouse ES cell line predominately differentiates to neural and myogenic cells "when cultivated in embryo-like structures", thereby reflecting a bias in the differentiation capacity of this mouse ES cell line Importantly, the effect of END2 co-culture on the formation of cardiomyocytes could not have been assessed as the authors noted that in contrast to other (mouse) ES cell lines, BLC6 cells "did not differentiate to cardiac cells". Page 91 of Rohwedel (1994).

Furthermore, Rohwedel (1994) also shows that the effect of co-culture with END2 cells actually inhibits formation of skeletal muscle (p 88), a derivative of mesoderm, in embryoid bodies. This teaching of Rohwedel (1994) would have discouraged the skilled artisan from using the END2 cell line for the promotion of the formation of mesoderm and subsequent cardiomycocyte and vascular endothelial cell lineages.

Consideration of the cited art in combination

Those skilled in the art would not have been motivated to use the co-culture system of Amit in the first instance, because an embryonic cell type is used therein for maintaining hES cells in a pluripotent, undifferentiated state to prevent their "loss to differentiation", rather than for directed differentiation of hES cell in the present invention. Further, the teaching of Amit would also negate any expectation of success, even if one were to attempt to coculture the hES cells with an embryonic cell type, because the embryonic cell type used in Amit

maintains the undifferentiated state of a hES cell, rather than causing directed differentiation of hES cells to vascular endothelial and cardiomyocyte cell types.

The teachings of Rohwedel (1994) relate to the formation of skeletal muscle cells from mouse ES (or EC) cells, not to cardiomyocytes from human ES cells. Moreover, a co-culture with END2 cells is taught to be inhibitory to myocyte formation, further removing any motivation or reasonable expectation of success the skilled artisan would have had in attempting the claimed invention.

Furthermore, consistent with Applicant's previous submission regarding the differing effect of the differentiation agents on mouse ES cells vs. human ES cells, the effect of RA at concentrations that result in cardiomyocyte differentiation in mouse p19 EC cells is toxic to human ES cells (see Xu et al. (2002), discussed above). Therefore, consistent with Applicants' submission in the previous Response, there are significant distinctions between ES and EC cells of the same species, and also between human and mouse ES cells, such that the differentiation response of human ES cells could not have been predicted based on the responses from mouse EC or ES cells.

Applicants note that several basic factual inquires must be made in order to determine the obviousness or non-obviousness of claims of a patent application under 35 U.S.C. § 103. These factual inquiries, set forth in *Graham v. John Deere Co.*, and require the Office to:

(1) Determine the scope and content of the prior art;

(2) Ascertain the differences between the prior art and the claims in issue;

(3) Resolve the level of ordinary skill in the pertinent art; and

(4) Evaluate evidence of secondary considerations.

The obviousness or nonobviousness of the claimed invention is then evaluated in view of the results of these inquiries. *Graeme v John Deere Co.*, 383 US 1,148 USPQ 459 (1966); see also *KSR International Co. v Teleflex Inc.*, 550 US 82 USPQ 2d 1385 (2007). Indeed, to establish a *prima facie* case of obviousness, the Office must make a determination whether the claimed invention "as a whole" would have been obvious at that time to that person. "The key to supporting any rejection under 35 U.S.C. § 103 is the clear articulation of the reason(s) why the claimed invention would have been obvious." MPEP 2142. Moreover, each prior art reference relied upon in a rejection "must be considered in its entirety, i.e., as a whole, including portions that would lead away.

Applicants respectfully submit that the Office has failed to establish a *prima facie* case of obviousness in the first instance based on a factual analysis according to *Graham v. John Deere Co.*

(1) The scope of the claims is such that the claims are directed to the induction of differentiation of human ES cells to mesoderm in the presence of embryonic cells or extracellular medium of the embryonic cells.

(2) The prior art provided relates to the use of mouse ES or EC cells. The art has a clear recognition of the distinctions between human ES cells and mouse EC or ES cells. The art reflects the unpredictable nature of the field, because the observations made with mouse ES and EC cells did not apply to human ES cells.

(3) The level of ordinary skill in the art was reflected by the art, namely, the understanding that human ES cells were different from mouse ES cells and that ES cells were different from EC cells.

(4) As evidence of secondary considerations, some of the art cited by the

Examiner contains disclosures that would teach away from the claimed invention. For example, feeder layers were used to maintain the ES cells in an undifferentiated state, rather than to cause or direct differentiation. END2 cells were shown to inhibit formation of myocyte, rather than to promote differentiation. The data available at the time relate to the evaluation of mouse EC cells. Data relating to hES cells was limited and the knowledge available at the time for directed differentiation was limited to spontaneous differentiation.

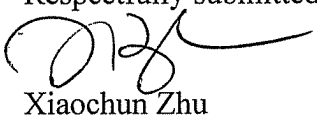
Therefore, Applicants respectfully submit that under the analytical framework set forth in *Graham*, the Examiner has failed to establish a *prima facie* obvious case. Moreover, none of the citations provide any teaching, suggestion or motivation that would have lead one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention.

Applicants respectfully submit that the present invention is not obvious over the cited combination of art, and the results provided are unexpected. Accordingly, withdrawal of the obviousness rejection is respectfully requested.

Conclusion

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



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